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Novel superabsorbent biosensor nanohydrogel based on gum tragacanth polysaccharide for optical detection of glucose

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ABSTRACT

Effective biosensor devices for detection of glucose based on carbohydrate polymer nanohydrogels have been scarcely reported to date. In an attempt to construct a more functional device for detection of blood glucose, CdTe quantum dots (QDs) and glucose oxidase (GOx) were immobilized within the well functionalized superabsorbent nanohydrogel prepared from the nanoparticles (<50 nm) of gum tragacanth (GT) polysaccharide. The biosensor was able to perform enzyme-catalyzed oxidation of glucose to produce H_2O_2, a product that subsequently quenched the fluorescence emission. The quenching rate depends on the glucose concentration, the detection limit was 0.5 mM, and the Michaelis–Menten constant (K_M) was an excellent value of 2.2 mM. The prepared biosensor was used for the enzymatic detection of blood glucose concentration in the real serum samples which exhibited satisfactory reproducibility and accuracy. Therefore, the high immobilization for QDs and GOx in this superabsorbent nanohydrogel matrix can be a suitable diagnostic device for biomedical applications.

1. Introduction

The modern design of various responsive efficient platforms with the utilization of different structures and mechanisms are required to tackle unmet clinical problems in diagnosing diseases. The fluorescent semiconductor quantum dots (QDs) are one of the most exciting fields in applications such as biological imaging and optical sensors [1,2]. The remarkable optical properties such as size-controlled fluorescence properties, high quantum yield, and stability make QDs suitable sensing materials for the sensitive and rapid detection of various molecules including metal ions [3] and phenol derivatives [4] to biomolecules such as glucose [5–7]. Neat and untreated QDs do not have specific recognition toward the detected objects [8]. Therefore, for the selective detection of various analytes, QDs must be coupled with different types of biomolecules including antibodies, nucleic acids, and enzymes. It was demonstrated for the first time in 1948 that glucose oxidase (GOx) catalyzes the aerobic oxidation of glucose to H_2O_2 and gluconic acid [9]. There is an excellent overview of the production and applications of GOx in the literature [10]. GOx is a dimeric glycoprotein composed of two identical polypeptide chains of approximately equal molecular weight covalently linked together by disulfide bonds. GOx is an oxidoreductase using O_2 as an electron acceptor to catalyze the oxidation of β-D-glucose to H_2O_2 and D-glucono-δ-lactone which then hydrolyzes to gluconic acid in a non-enzymatic step. GOx has found several commercial applications including in biosensors for the detection of free glucose in blood and urine [5]. Glucose is one of the most common analytes that the measurement of its concentration in blood is critical for the diagnosis of diabetes. There are studies on the optical biosensor based on hydrogels [11,12] where most of them have been developed either using QDs [13] or enzyme [14,15]. However, these QDs based glucose-detecting biosensors may show good sensitivity but most of them were restricted to solution-based sensing assays. Also, the hydrogel used as the biosensing platform must possess a large surface area, chemical, and mechanical stability [16,17]. Hydrogels are typical soft polymeric materials with the semi-wet three-dimensional network which can provide near-physiological conditions for enzymes to carry out their biological functions [6,18]. Polysaccharide hydrogels are preferred because of their local availability, low-cost, biodegradable, biocompatible, chemical stability, and easy modification [19,20]. One of the most abundant, eco-friendly, not-toxic, and the low-cost polysaccharide is Gum Tragacanth (GT) native to the regions of the eastern Mediterranean, Southwest of Asia, and particularly in the Zagros Mountains region in Iran. GT from different locations have different levels of water-soluble tragacanthin (75%), consisting mainly galacturonic acid, and water-swellable bassorin (25%), consisting mainly L-fucose, fractions [21–23]. However, the main backbone of GT macromolecular carbohydrate contains linear chains of 1,4-linked α-D-galacturonic acid residues. GT macromolecules readily form transparent hydrogel that can entrap functional molecules and is potentially an attractive

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polysaccharide for development of novel sensing systems for biosensor applications.

Herein, we sought to develop a novel biosensing platform from GT nanohydrogel by immobilizing CdTe quantum dots (QDs) and GOx enzyme via a facile route of chemical cross-linking process. The superabsorbent nanohydrogel was prepared by grafting acrylic acid (AA) on GT macromolecules using *N*,*N*′-methylenebisacrylamide (MBA) as a cross-linker and potassium persulfate (KPS) as the redox initiator in a radical polymerization. Incorporation of CdTe QDs and GOx enzyme in this superabsorbent nanohydrogel was used to study the enzymatic conversion of the glucose into the quenching agent which simultaneously reduces the fluorescence intensity of QDs entrapped inside the GT nanohydrogel network. The amine groups in the GOx enzyme structure can interact with the carboxylic acid groups in the macromolecular network forming stable linkages within the nanohydrogel. Therefore, the most important advantage of this work is the immobilization of QDs and GOx inside the superabsorbent nanohydrogel of a polysaccharide, as a highly sensitive glucose biosensor, which has not been explored before. Meanwhile, GT is a local and inexpensive polysaccharide with many advantages such as biocompatibility, biodegradability, and non-toxic. We hope that such new strategy and selected architecture may assist us in rationally designing new, effective, and functional GT-based nanohydrogels for advanced sensory nanocomposite format.

2. Experimental

2.1. Materials

The high-quality Gum Tragacanth (GT) of Zagros Mountains region has been purchased from the local pharmaceutical shop in Sanandaj (Iran). GT macromolecular carbohydrate contains linear chains of 1,4-linked α-D-galacturonic acid residues in its main backbone. To overcome water solubility restrictions, the hydroxyl and carboxylic acid groups in GT macromolecules provide the desired positions for grafting on with different monomers bearing functional chelating groups [24,25]. Glucose oxidase (GOx, from Aspergillusniger type II), D-glucose, Acrylic acid (AA), *N*,*N*′-methylenebisacrylamide (MBA), cadmium chloride ($CdCl_2 \cdot 2.5H_2O > 98\%$), sodium borohydride ($NaBH_4$, ~98%), tellurium powder (−200 mesh, 99.8%), potassium persulfate, phosphate Buffered Saline (PBS, pH = 7.4) and mercapto acetic acid (MAA) ($HS\text{-}CH_2COOH$) were purchased from Sigma-Aldrich Co. (USA). All other chemicals used in this study were analytical grade and used without further purification.

2.2. Synthesis

2.2.1. Synthesis of CdTe QDs

CdTe QDs has been used in this study due to the availability of the starting materials for the synthesis. CdTe QDs have a band gap energy of 1.52 eV and their particles size can be controlled according to the quantum confinement of charge carriers suitable for emitting in the visible spectral range if. Also, CdTe QDs can be produced in the aqueous phase more easily [26]. Water-soluble CdTe QDs was synthesized according to the previously reported method [27]. Briefly, a mixture of 1.6 mmol tellurium powder, 4.2 mmol $NaBH_4$ and 26.8 mL deionized water in a 50 mL three-necked flask equipped with a reflux condenser and a nitrogen gas inlet tube was refluxed under a stream of N_2 at 100 °C for 1.5 h and constant stirring. After cooling, the obtained clear-purple solution (NaHTe) was transferred to a dropping funnel. The cadmium thiolate was prepared by mixing 6.4 mmol $CdCl_2$ and MAA as a stabilizer (molar ratio 1:6) in 160 mL deionized water. The pH was adjusted to 5 by adding aliquots of a 3 M NaOH aqueous solution and then was heated up to 70 °C. For the nanoparticles synthesis, NaHTe solution was added drop-wise into this hot Cd solution to start the reaction under vigorous stirring and N_2 flow. After the injection, the temperature was increased to 150 °C for 4hto let the growth of CdTe semiconductor nanocrystals with green emission.

2.2.2. Preparation of GT nanoparticles (NGT)

GT nanoparticles, for the first time, were prepared by sieving GT to remove dust and small particles, purified with ethanol extraction, dried under vacuum for 48 h, and kept in desiccators before use. Purified 0.5 g GT was dispersed in 60 mL distilled water and the pH was adjusted to 3.7 by adding aliquots of a 2 M HCl aqueous solution. This suspension was sonicated by using Hielscher Ultrasonic (GmbH, UP200H Germany) equipped with a converter/transducer and titanium oscillator (horn), 3 mm, operating at 241 kHz with a maximum power output of 200 W. Then the sonicated suspension was stirred in a 100 mL round-bottomed flask equipped with a reflux condenser and a magnetic stir at 50 °C for 24 h. Finally, the clear and light viscose solution was poured in acetone to complete precipitation. The filtered precipitate was dried under vacuum for 24 h at 50 °C. FT-IR analysis was carried out on a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany) to confirm the structure of NGT. Surface morphology of the NGT and its size was examined by field emission scanning electron microscopy (FE-SEM, HITACHI S-4160).

2.2.3. Preparation of GT nanohydrogels [GT-PAA-MBA-PAA-GT]

The modification of NGT with acrylic acid (AA) monomer and MBA cross-linker was carried out in the presence of potassium persulfate (KPS) as the redox initiator. 0.5 g dried NGT was dispersed in 50 mL distilled water in a 250 mL three-necked flask and stirred for 30 min to attain homogenous mixture. The initiator solution (0.05 g KPS in 2 mL distilled water) was added to the mixture and stirred at 70 °C for 0.5 h. Subsequently, AA (0–5 mL) and MBA (0.1–0.3 g) were added to the solution and stirred at 70 °C for 4 h. A continuous supply of nitrogen was maintained throughout the reaction period. To complete the precipitation, the mixture was poured in acetone and then the filtered precipitate was extracted for 24 h in a "Soxhlet apparatus" using water. The product was dried under vacuum at 50 °C for 24 h. FT-IR analysis and FE-SEM images were carried out to investigate the structure and surface morphology of nanohydrogel, respectively.

2.3. Measurements

2.3.1. Swelling behavior of nanohydrogel

It is well known that swelling behavior of a nanohydrogel can be affected by factors such as cross-linker content, pH of the swelling medium, and contact time. The swelling behavior of the nanohydrogels was investigated in deionized water at room temperature and various pH values (3, 7 and 10) using a gravimetric method. The weighted dried nanohydrogels were placed in a beaker containing 50 mL distilled water. The swollen nanohydrogels were removed periodically and wiped off with a tissue paper and then being weighed. The swelling procedure was repeated until the sample has reached a constant weight. The swelling ratio (SR) percentage was calculated as a function of time using Eq. (1):

$$SR\% = \left(\frac{W_t - W_d}{W_d}\right) \times 100 \quad (1)$$

where W_d is the weight of the dried gel before swelling and W_t is the weight of the swollen gel at a certain time. Each recorded data is an average value of three individual sample readings.

To estimate gel content, the nanohydrogels with the most swelling were selected. In this way, dried nanohydrogels were weighed before

Table 1
Feed composition and swelling behavior of the nanohydrogels at pH 7.

Entry	NGT (g)	MBA (g)	AAc (ml)	Swelling ratio %
H-1	0.5	0.1	0	8
H-2	0.5	0.1	1	13
H-3	0.5	0.1	2	64
H-4	0.5	0.1	3	77
H-5	0.5	0.1	4	150
H-6	0.5	0.1	5	63
H-7	0.5	0.2	0	12
H-8	0.5	0.2	1	20
H-9	0.5	0.2	2	163
H-10	0.5	0.2	3	316
H-11	0.5	0.2	4	93
H-12	0.5	0.2	5	45
H-13	0.5	0.3	0	9
H-14	0.5	0.3	1	15
H-15	0.5	0.3	2	253
H-16	0.5	0.3	3	219
H-17	0.5	0.3	4	119
H-18	0.5	0.3	5	69

(W_0) and after (W_d) the Soxhlet extraction. The gel fraction was calculated using Eq. (2).

$$\text{Gel fraction } (\%) = \frac{W_d}{W_0} \times 100 \quad (2)$$

2.3.2. QDs loading and leakage and stability tests

The nanohydrogels with the highest swelling ratio were selected for QDs loading. 1 mL QDs solution in a mixture of 0.1 g nanohydrogels, 1 mL NaOH aqueous solution (pH = 10), and 2 mL deionized water was kept at room temperature for 30 min. The uniform dispersion of QDs in nanohydrogels was investigated by fluorescent microscope (Smart Flu, Canada Smart) and FE-SEM. The leakage of QDs was investigated by adding 2 mL deionized water to the nanohydrogel containing CdTe QDs. The supernatant was taken out at predetermined time intervals and replaced with fresh deionized water. The fluorescence spectra of the collected supernatant and the initial QDs solution were recorded with measuring at 555 nm using the FP-8300 spectrofluorometer (JASCO. Japan). Also, the stability of the QDs inside nanohydrogel was evaluated by leaving the sample at room temperature and 4 °C for 45 days and then measuring the fluorescence intensity by using spectrofluorometer.

2.3.3. Glucose oxidase (GOx) loading and the leakage test

The nanohydrogels with the highest water swell-ability were used for enzyme loading using the swelling-diffusion method. A solution of 1 mg GOx enzyme in 1 mL PBS (pH = 7.4) was added in a mixture of 0.1 g nanohydrogels and 2 mL deionized water and stirred for 30 min at room temperature. For the enzyme leakage test, the same procedure as used in the QDs leakage test was used. The amount of enzyme leakage was determined using the Stern-Volmer equation. Toward this end, the calibration curve of H_2O_2 was made using various concentrations of H_2O_2 (0, 0.01, 0.1, 0.5, 1, 3, 5, 7 and 10 mM) and monitoring the quenching degree of fluorescence of the QDs at 555 nm. The fluorescence spectra of solutions containing 3 mL of various concentrations of H_2O_2 and 1 mL QDs solution were recorded. The supernatant was assayed with a 3 mM glucose solution that included 1 mL QDs. The activity of the encapsulated GOx was determined by measuring at 555 nm using spectrofluorometer.

2.3.4. Glucose detection and GOx stability

To detect specific target substrates such as glucose, samples of nanohydrogels containing QDs and GOx enzyme were prepared in the separate beakers and their fluorescence spectra were recorded before and after exposure to the solution of various concentrations of glucose (0–10 mM). The degree of fluorescence quenching of the QDs-encapsulated nanohydrogels was used to determine the concentration of the glucose. For the GOx enzyme stability evaluation, the nanohydrogels sample containing GOx was kept at room temperature and 4 °C for 72 h. Then assayed with certain concentrations of substrate and their sensitivity decrements were determined by spectrofluorometer.

2.3.5. Detection of glucose in the real blood sample

The blood samples with a certain glucose concentration were given by a local laboratory. Each real sample was placed in a beaker of nanohydrogels loaded with QDs and GOx enzyme for 20 min. The glucose concentrations were determined by the calibration curve and compared with the real values.

3. Results and discussion

One of the principal goals in the present study was to develop a high-sensitivity biosensor system to determine blood glucose. To reach this goal, novel superabsorbent nanohydrogels based on gum tragacanth (GT) were prepared and used for loading with QDs and GOx enzyme.

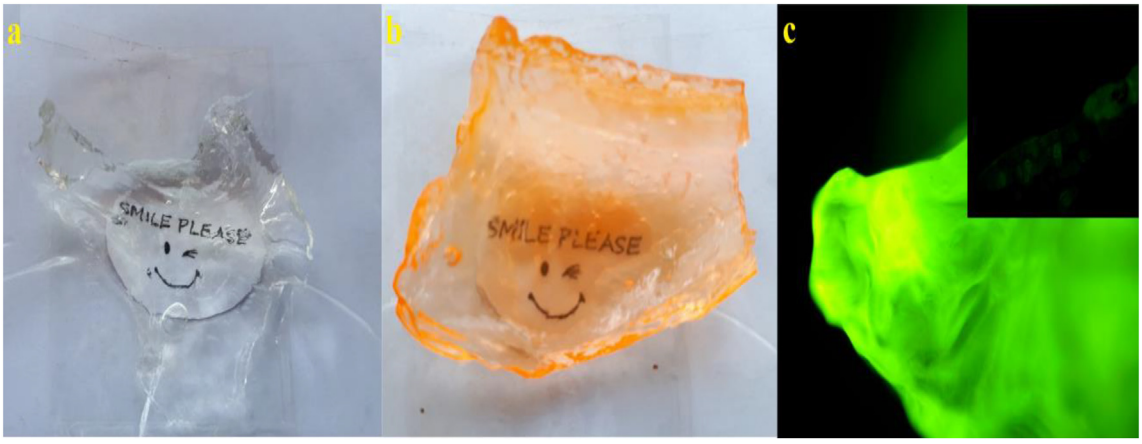

Fig. 1. For the transparency confirmation, photos of nanohydrogel were taken without QDs (a) and with encapsulated QDs (b). Photo was taken by the fluorescence microscopy of the nanohydrogel containing QDs (c), the inset shows the photo of the same sample after addition H_2O_2.

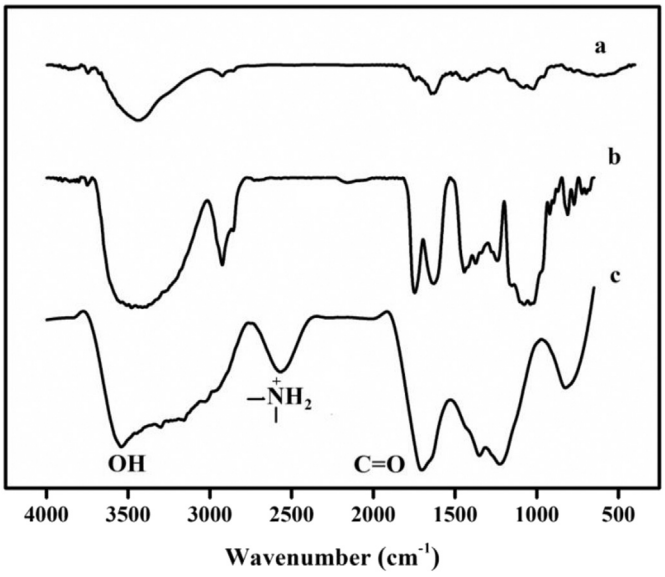

Fig. 2. FT-IR spectra of: a) GT (reprinted with permission from ref. Rahmani et al. [28]), b) NGT, and c) GT nanohydrogel.

3.1. Synthesis and characterization of nanohydrogels

GT nanoparticles (NGT) were prepared with a simple method by using pH adjusting and sonicating. Different nanohydrogels were synthesized by using various mass ratios of MBA and AA, as shown in Table 1. The swelling ratio increased initially with increasing the amount of MBA as cross-linker and then started to decrease as a result of the formation of the highly cross-linked network. AA monomer increased the hydrophilicity and water absorbency of the nanohydrogels due to the strongly ionizable carboxylic acid groups. However, a further increase in AA content led to a decrease in swelling ratio due to the increased hydrogen bonding between carboxylic groups. Finally, the nanohydrogel synthesized by using 0.2 g cross-linker and 3 mL AA (H-10) was mechanically strong enough and also had the high swelling capacity (316%) with enough gel fraction (78%) and superior transparency as shown in Fig. 1a and b. Also, the swelling behavior of the nanohydrogels was determined in pH 3 and 10 and the swelling ratios were 103 and 520%, respectively. Therefore, the superabsorbent nanohydrogels H-10 was selected for encapsulation of CdTe QDs and GOx enzyme and used for detection of glucose. The prepared hydrogels were extracted in a Soxhlet using distilled water and dried in the vacuum oven before grinding into the fine particles, which was mixed with QDs solution and enzyme solution in PBS for fluorescence measurement. The ground particles show the maximum swelling due to the increase in the surface area.

Comparison of the FT-IR spectra of GT (Fig. 2a) and NGT (Fig. 2b) confirmed that the peak intensity for NGT has increased without changes in the structure of GT. In case of GT, there are absorption bands at 3749–3070, 985–1220, 3000–2800, 1743 and 1648, and 812 cm^{-1} due to the stretching vibration of O—H, C—O, C—H, asymmetric stretching of C═O in galacturonic acid and carboxylate groups, and pyranose ring vibration, respectively [28]. Nanohydrogel with superabsorbency properties was prepared from radical graft copolymerization of AA and MBA onto NGT backbones in the presence of potassium persulfate (KPS) as a redox initiator. Therefore, the existence

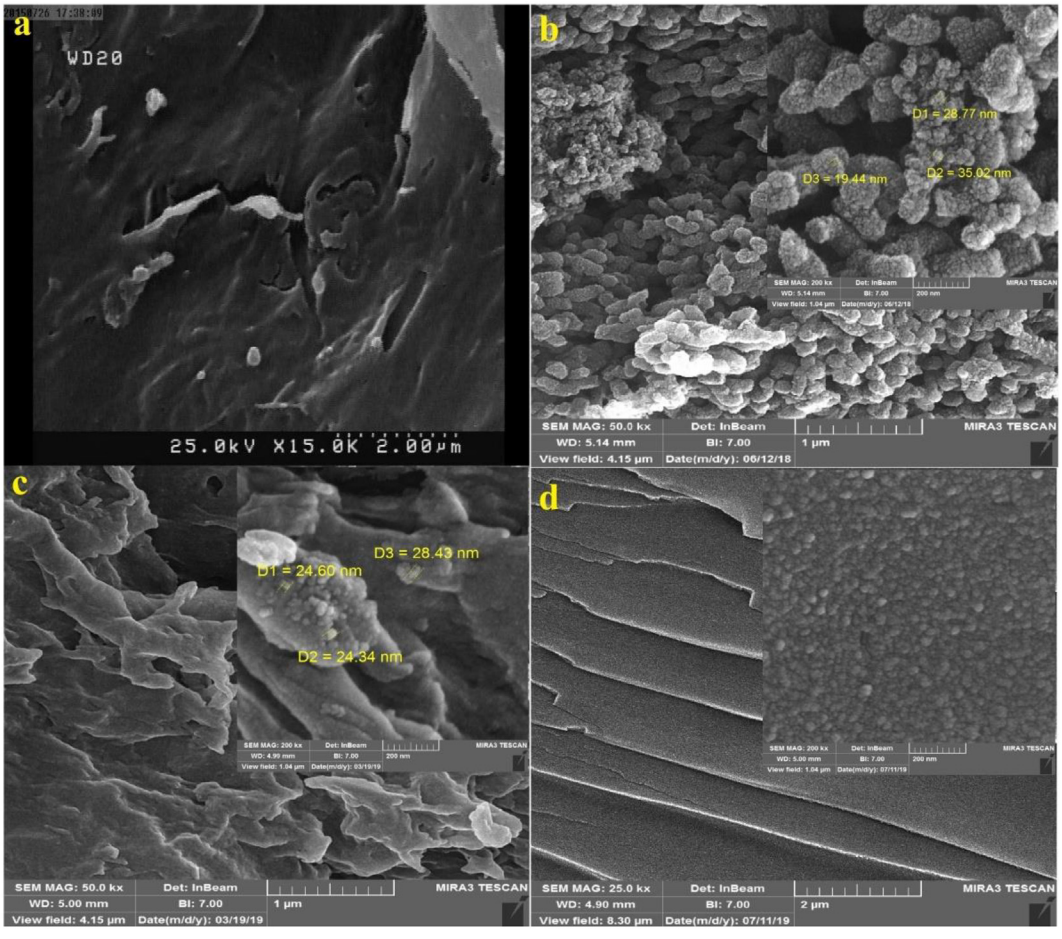

Fig. 3. FE-SEM images of: a) GT (reprinted with permission from ref. Rahmani et al. [28]), b) NGT, c) GT nanohydrogel, and d) QDs encapsulated in GT nanohydrogel.

of the ionized charges in the nanohydrogel network increases the hydrophilic nature and the electrostatic repulsion between the polymer chains leading to a high degree of swelling [29]. Also, the three-dimensional structure and the presence of many functional groups provide the physical and intermolecular interaction to stabilize the entrapped CdTe QDs and GOx enzyme in the nanohydrogel matrix. The FT-IR spectrum of the nanohydrogel in Fig. 2c shows the absorption band of the carbonyl group at the 1600–1800 cm^{-1}region which, in comparison with the spectrum of NGT, is more intense and wider indicating the increase in the number and type of carbonyl groups. The characteristic absorption band of protonated N—H stretching of MBA is observed at 2500–2600 cm^{-1}, the absorption band of C—H stretching vibration (2900–3500 cm^{-1}) is not seen due to overlapping by the acid group.

The surface morphology of GT and NGT were investigated by using FESEM microscope. In comparison to the smooth and uniform surface of GT, as shown in Fig. 3a [28], the FESEM image of the prepared NGT (Fig. 3b) presents non-smooth and globular particles with sizes <50 nm. Also, the FESEM image of the nanohydrogel sample (in Fig. 3c) shows heterogeneous structure with rough surface which can be due to the graft copolymerization and formation of three-dimensional network.

3.2. QDs loading and leakage and stability tests

To utilize the prepared nanohydrogel as a biosensor platform, the incorporated QDs and enzyme within the nanohydrogel should be well retained during the detection process. Therefore, possible leakage of QDs and enzymes from the nanohydrogel was investigated using multiple diffusion tests as explained in Sections 2.3.2 and 2.3.3. The changes of fluorescence intensity of the collected supernatants at 555 nm were recorded during incubation and the degree of QDs leakage was calculated based on the fluorescence intensity of the initial QDs used for the loading. The total QDs leakage from the nanohydrogel was ~1.48% after 2 h (Fig. 4a) indicating the strong adherence of QDs in the matrix of the nano-sized three-dimensional network. Also, the FESEM image in Fig. 3d shows the uniform dispersion of QDs in the nanohydrogel network. Additional confirmation is the photo in Fig. 1c showing intense and homogeneous fluorescence emission throughout the nanohydrogel sample. Also, the inset in Fig. 1c shows the fluorescence quenching photo of the same sample after H_2O_2 addition. To investigate the stability of the entrapped QDs, the changes in the fluorescence intensity of the nanohydrogels containing QDs were kept at room temperature and 4 °C for up to 45 days was measured by spectrofluorometer. As shown in Fig. 4b, the fluorescence intensity of QDs has not changed significantly after 45 days storage at 4 °C while the fluorescence intensity of the sample stored at ambient temperature decreased ~17% after 45 days storage.

3.3. Encapsulation efficiency and leakage of GOx enzyme

In the following, the possibility of GO_X release from the nanohydrogel network was investigated. The enzyme leakage and subsequently enzyme activity were measured by the Stern-Volmer equation that is both economically feasible and consistent with green chemistry and can be a substitute for the calorimetric method [5,30].

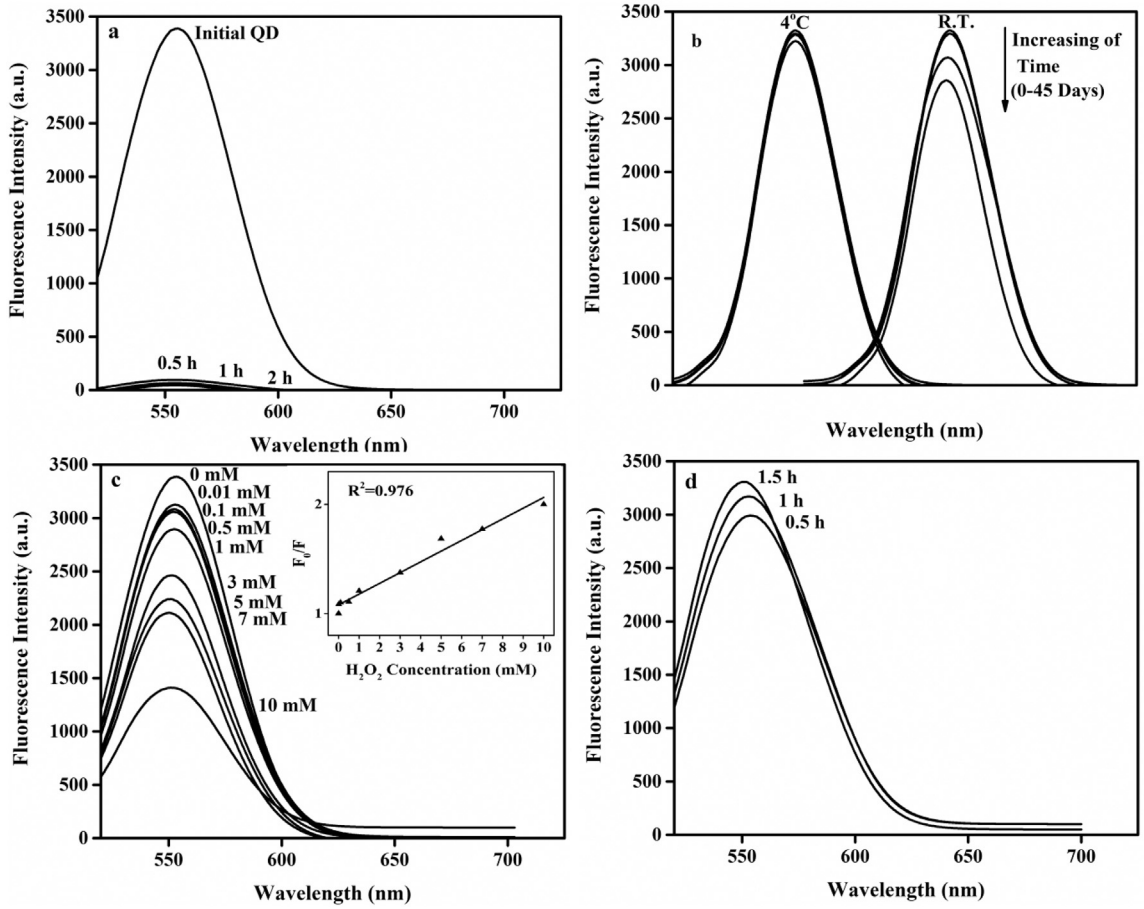

Fig. 4. a) Leakage test of QDs, b) stability of QDs within the nanohydrogel was evaluated by measuring fluorescence intensity at 4 °C and R.T. after 45 days. Leakage test of enzyme: c) the effect of H_2O_2concentration on the fluorescence intensity of nanohydrogel, the inset shows the Stern-Volmer curve (calibration curve), and d) fluorescence intensity of supernatants that was reacted with a certain glucose concentration at different times.

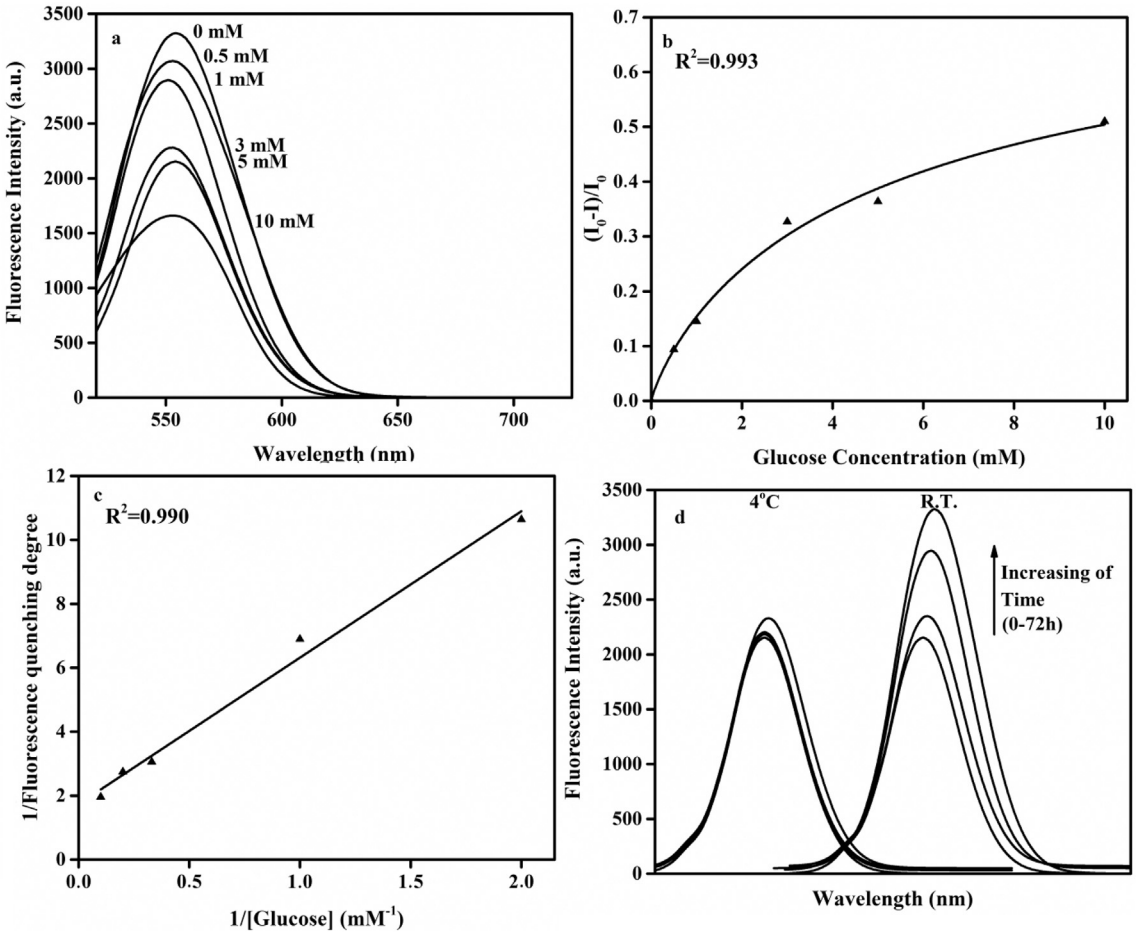

Fig. 5. Detection of glucose using GT nanohydrogel incorporated with QDs and GOx enzyme. a) Fluorescence spectra of GT nanohydrogel containing CdTe QDs and GOx at different glucose concentrations, b) changes of fluorescence intensity for the resulting nanohydrogel at 555 nm at different glucose concentrations, c) lineweaver–Burk plot based on the fluorescence spectra of the nanohydrogel at different glucose concentrations, and d) stability of enzyme within the nanohydrogel was evaluated by measuring fluorescence intensity at 4 °C and R.T. after 72 h.

The degree of the enzyme leakage was calculated as described in Section 2.3.3. The results in Fig. 4c show the effect of H_2O_2 concentration on the fluorescence intensity of QDs in the nanohydrogel. As can be seen in this figure, fluorescence intensity of QDs decreases sharply with increasing H_2O_2 concentration. Therefore, the results for the enzyme activity of supernatant mixed with a glucose solution containing QDs showed a very trace amount of enzyme leakage, as observed in Fig. 4d. The enzyme molecules can be immobilized within the nanohydrogel through interaction of the amine groups in the dimeric protein of GOx with the carboxylic acid groups in the nanohydrogel.

3.4. Detection of glucose and stability of enzyme

Glucose exists in the cyclic hemiacetal form of β-D-glucopyranose and α-D-glucopyranose. The GOx enzyme catalyzes the oxidation of β-D-glucopyranose to hydrogen peroxide and gluconic acid. The equilibrium between the α and β anomers is driven toward the β side as it is consumed in the reaction. It is well known that the H_2O_2 which is produced via enzymatic-catalyzed oxidation of glucose acts as an electron acceptor on the surface of CdTe and quenches the fluorescence intensity of QDs. The suitability of the nanohydrogels for biosensing applications is evaluated by the efficient embedment of the enzyme receptors via physical entrapment without losing their structures and/or reducing their activities significantly. To prove this claim for the present biosensor, the GOx enzyme as a bioreceptor was incorporated within the fluorescent nanohydrogel and applied for detection of the glucose level in solution.

According to the obtained results, the degree of quenching was well correlated in the region 0.5–10 mM of glucose concentration (Fig. 5a), which fully covers the range of 3.5–6.5 mM required for the diagnosis of diabetes [31]. As shown in Fig. 5a, the fluorescence intensity of CdTe QDs in the nanohydrogel decreased proportionately after injection of each portion of glucose concentration. This indicates the gradual swelling of the nanohydrogel in the glucose solution, and diffusion of glucose into the nanohydrogel matrix which react with the O_2 under catalytic activity of the embedded GOx and lead to the production of H_2O_2. The activity of the enzyme was evaluated by Michaelis–Menten constant (K_M) obtained from the Michaelis–Menten saturation curve (Fig. 5b) and Lineweaver–Burk plot (Fig. 5c). The obtained K_M value of 2.2 mM is much lower than K_M values previously reported for other gel-like materials such as silica gel ($K_M = 20.3$) [32], carbon nanotubes/chitosan matrix ($K_M = 8.2$) [33] alginate beads ($K_M = 30$) [34] and peptide nanohydrogel ($K_M = 3.12$) [5]. The small value of K_M indicates that the enzyme embedded in the nanohydrogel has a high affinity for glucose. This can be due to the high loading capacity of the superabsorbent nanohydrogel and the enhancement of the mass transport of substrates and products. To investigate the stability of the entrapped enzyme, the changes in the fluorescence intensity of the nanohydrogels containing enzyme stored at room temperature and 4 °C for 24, 48 and 72 h were investigated by spectrofluorometer. As shown in Fig. 5d, the fluorescence intensity of the QDs in the nanohydrogels

Table 2
Glucose concentration (mM) in the real blood sample was measured by different methods.

	Spectrofluorometry method	Spectrophotometry method[a]
1	6.42	6.5
2	5.85	5.9

[a] Glucose oxidase and peroxidase enzymes and organic dyes are used to detect glucose.

stored at 4 °C for 72 h changed slightly showing the stability of the enzyme at this temperature. While the fluorescence intensity of the QDs in nanhydrogel stored at ambient temperature has increased greatly showing the instability of the enzyme with time at room temperature. Many enzymatic processes including those catalyzed by GOx, alcohol oxidase (AOx), lysine oxidase (LOx), and chlorine oxidase (COx) produce H_2O_2 during oxidation-reduction reactions. Therefore, embedding QDs with these enzymes in the as-prepared nanohydrogel can also yield a promising biosensor for many oxidase enzyme substrates.

3.5. Determination of glucose in the real blood samples

The feasibility of the prepared biosensor for the analysis of biological fluids was evaluated by detection of glucose using two real blood samples without pretreatment. The glucose concentrations of the real blood samples obtained by using the as-prepared nanohydrogel biosensor were in a reasonable agreement with the reported values, as shown in Table 2.

4. Conclusion

A novel superabsorbent biosensor has been successfully synthesized from gum tragacanth (GT) polysaccharide, AA monomer, and MBA cross-linker in a radical polymerization. The main advantage of this approach is the formation of a functionalized superabsorbent nanohydrogel which provides QDs and GOx encased within a cross-linked network with minimal leakage, responsible for good operational stability. The swelling ratio, mechanical stability, and the transparency of the hydrogels which affect the percentage of enzyme leakage, encapsulation efficiency, and linear range and sensitivity of glucose determination, can be adjusted by controlling the weight ratios of AA and MBA. The prepared biosensor has been used for the optical detection and determination of glucose in artificial and real media with satisfactory reproducibility and accuracy. It has been demonstrated that the detection of glucose diffused inside the nanohydrogel is based on the quenching ability of H_2O_2 produced by oxidase-catalyzed reactions in the glucose. Due to the strong immobilization of QDs and GOx in the nanohydrogel, the application of the biosensor developed in this fashion can be utilized in vivo and extended in practical biological environments. There is no limitation for the as-prepared biosensor, it is used not only for the H_2O_2-generating oxidase but can also be used on any other enzymatic-products quenching QDs fluorescence.

CRediT authorship contribution statement

Soudabeh Qasemi: Formal analysis, Data curation, Investigation, Software, Writing - original draft.**Mousa Ghaemy:**Methodology, Project Adminstration, Supervision, Validation, Writing - review & editing.

Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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